



# Neighbour–stranger discrimination by territorial male bullfrogs (*Rana catesbeiana*): I. Acoustic basis

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(Received 21 August 2000; initial acceptance 19 October 2000;  
final acceptance 10 April 2001; MS. number: A8863)

Some territorial animals discriminate among neighbours and strangers based on individual differences in acoustic signals. Male North American bullfrogs, *Rana catesbeiana*, display this form of discrimination based on individual variation in advertisement calls. In this study, we investigated the acoustic basis of neighbour–stranger discrimination to determine how individual identity might be encoded by particular properties of bullfrog advertisement calls. We analysed patterns of within-male and between-male variability in 1078 bullfrog advertisement calls recorded from 27 territorial males. All call properties that we examined varied significantly among males. However, fundamental frequency and dominant frequency showed the lowest within-male variation and the highest repeatability between two recording sessions, and both properties were highly correlated with the first canonical root from discriminant function analyses, which typically accounted for 70–80% of the variability between males. We suggest that neighbour–stranger discrimination in bullfrogs is partially mediated by between-male differences in the spectral or fine temporal properties of advertisement calls.

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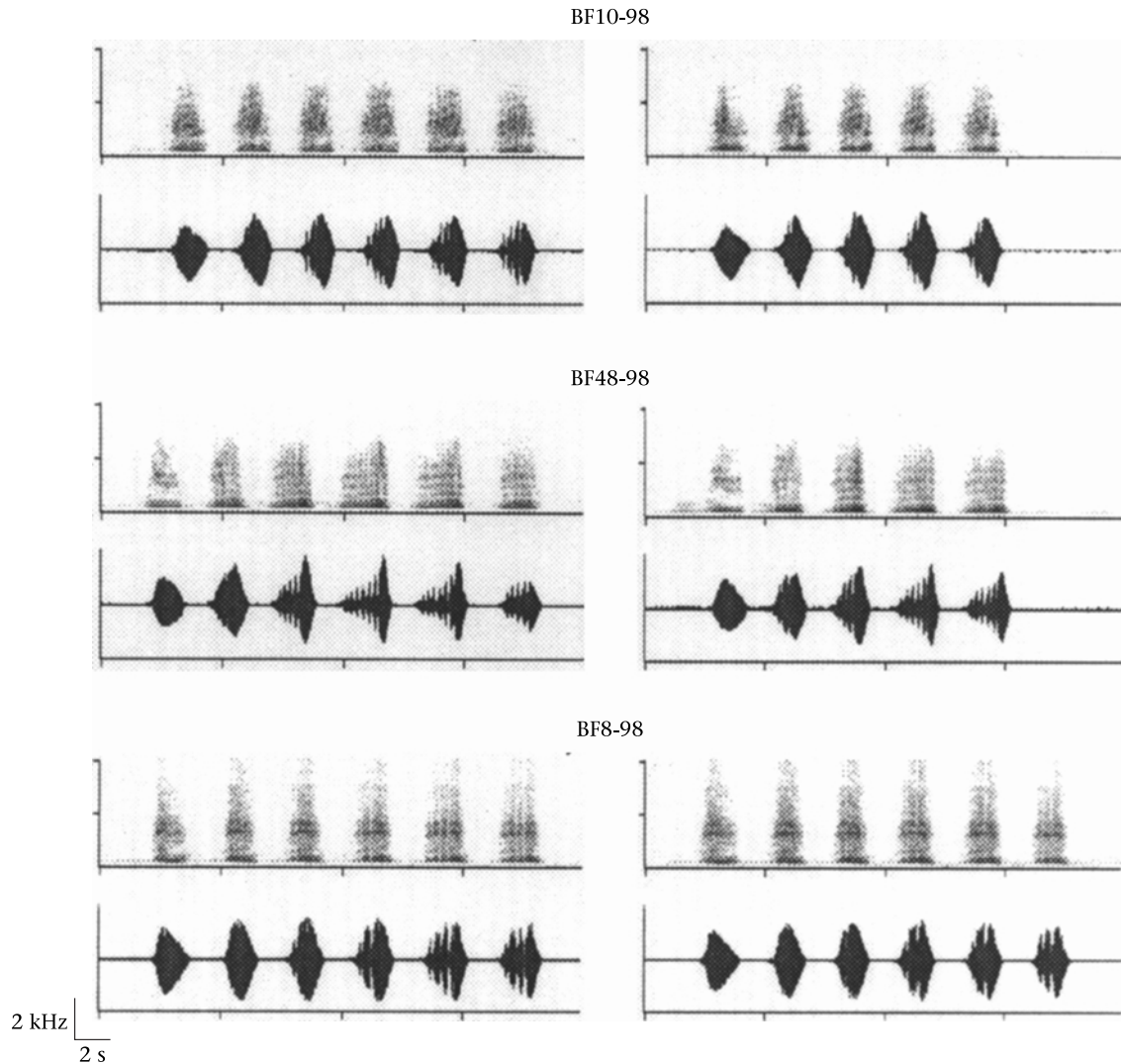
Territorial animals display a form of social recognition known as the ‘dear enemy’ phenomenon, in which they behave less aggressively towards familiar neighbours than towards unfamiliar animals (reviews in [Ydenberg et al. 1988](#); [Temeles 1994](#)). In many instances, discrimination between neighbours and strangers is mediated by a combination of acoustic communication signals and spatial cues ([Falls 1982](#); [Lambrechts & Dhondt 1995](#); [Stoddard 1996](#)). Although vocally mediated neighbour–stranger discrimination is well documented, relatively few studies have simultaneously and systematically examined individual variation in acoustic signals and the perceptual basis of recognition ([Brooks & Falls 1975](#); [Brown et al. 1988](#); [Nelson 1989](#)). Consequently, little is known about how territorial animals use acoustic signals to identify neighbours as familiar individuals, either within or across species.

We address this limitation in this study and in a companion paper ([Bee & Gerhardt 2001a](#)) by investigating the mechanisms of vocally mediated neighbour–stranger discrimination by territorial males of the North

American bullfrog, *Rana catesbeiana* (Anura, Ranidae). Neighbour–stranger discrimination based on vocalizations is known only from bullfrogs among anuran amphibians ([Davis 1987](#)), despite the fact that numerous frogs are territorial and highly vocal during their breeding seasons ([Wells 1977](#)). During a prolonged summer breeding season, male bullfrogs defend territories that females use as oviposition sites ([Howard 1978](#)). In a field playback experiment, [Davis \(1987\)](#) demonstrated that territorial male bullfrogs responded more aggressively to the advertisement calls of strangers than to those of neighbours when both calls were broadcast from within the neighbour’s territory, as in many territorial songbirds ([Falls 1982](#); [Lambrechts & Dhondt 1995](#); [Stoddard 1996](#)).

The purpose of the present study was to investigate the acoustic basis of neighbour–stranger discrimination in bullfrogs by determining how patterns of individual variation in advertisement calls give rise to individually distinct acoustic communication signals. We examined within-male and between-male variability in particular acoustic properties of bullfrog advertisement calls and used multivariate statistical analyses to investigate the potential of these properties to identify individuals. Our goal was to generate testable hypotheses about how the signal properties might function as acoustic cues for discrimination among territorial males. In our

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**Figure 1.** Sonograms and oscillograms of two multiple-note advertisement calls from each of three territorial male bullfrogs. The two calls for a male were taken from the same recording session. Bars indicate scale for frequency in kHz (vertical) and time in seconds (horizontal).

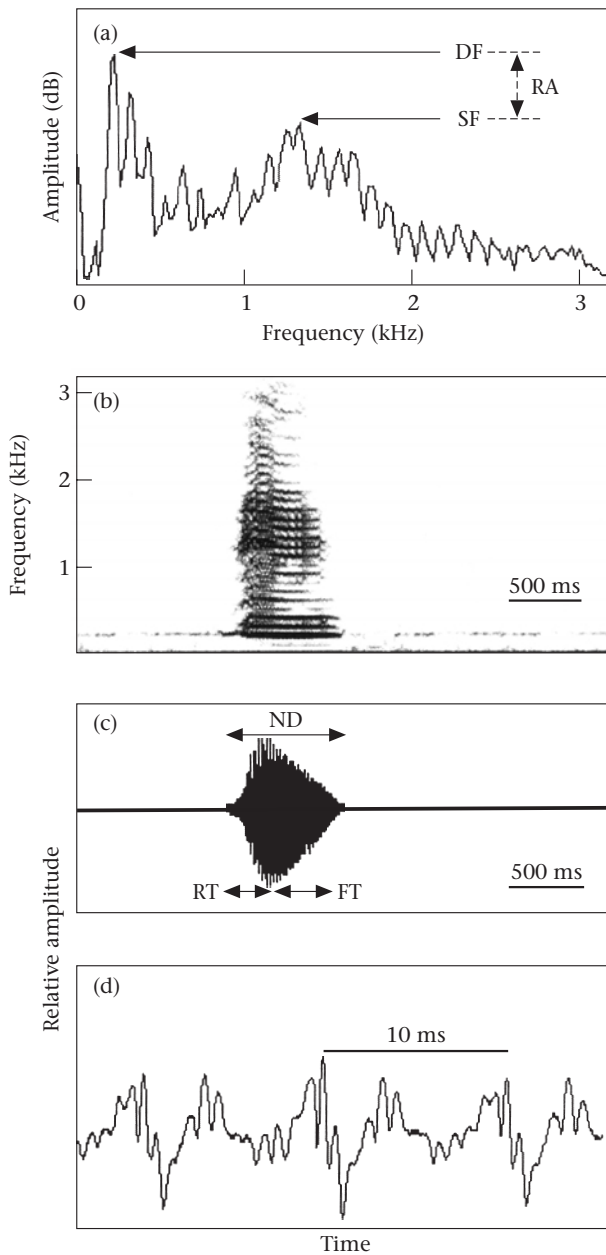
companion paper (Bee & Gerhardt 2001a), we demonstrate that male bullfrogs can perceive and learn about individually distinct signal properties as a result of repeated exposures across a territorial border.

## METHODS

### Recording Vocalizations

Between May and August, 1998, we recorded the vocalizations of male bullfrogs that held territories in ponds located in the Little Dixie Conservation Area (Callaway Co., Missouri, U.S.A.) and the Thomas Baskett Wildlife Area (Boone Co., Missouri, U.S.A.) (see Bee & Gerhardt 2001b for additional details). The most common vocalization heard during nightly bouts of chorusing is the advertisement call ('mating call' of Capranica 1965), which consists of one to 16 notes, or 'croaks' (Figs 1, 2). We recorded 1078 advertisement calls from 27 territorial male bullfrogs using a Sennheiser MKH 70 shotgun

microphone equipped with a Windtech SG-3 windscreens and mounted on a tripod placed 1–2 m from a subject and angled downward between 25° and 45° from parallel with the surface of the pond. Slight variation in the position of the microphone between recording sessions probably had negligible effects on between-male call differences (Boatright-Horowitz et al. 1999). We recorded calls onto digital audio tape using an HHb PDR-1000 DAT recorder (sampling rate=32 kHz) from a position 3–6 m away from the subject. We obtained one set of 20 consecutive calls from each male in each of two separate recording sessions (median inter-recording interval=2 days, range 1–16 days). For one male in each recording session, we obtained only 19 calls. Immediately after each recording of a frog, we determined the air and water temperatures at the frog's position to the nearest 0.1 °C. Recordings were made during active choruses, under ambient light conditions, between 2130 and 0530 hours, Central Daylight Time. On nights prior to those of recording, we captured each subject and measured its



**Figure 2.** (a) Power spectrum of an advertisement call note averaged over a 100-ms section from the middle of the note, beginning at the peak of the amplitude envelope. (b) Sonagram of a single-note call. (c) Oscillogram of the single-note call in (b). (d) Oscillogram of the middle of the call in (b) illustrating the fine temporal waveform. Dominant frequency (DF), secondary frequency (SF) and the relative amplitude (RA) were measured from power spectra as in (a). Note duration (ND), rise time (RT), fall time (FT) and note period were measured from oscillograms as in (c). Fundamental frequency ( $=1/\text{waveform periodicity}$ ) was determined by taking the reciprocal of the average period of five repetitions of the fine temporal waveform shown in (d). Horizontal bars indicate time scales in (b)–(d).

snout-vent length (SVL) and mass. Males were given individual toe clips on their hind feet, a cohort mark indicating the year on their fore feet, and a waistband and tag with an identifying number that allowed visual identification from a distance of 2–3 m.

### Ethical note

Individuals had to be marked for identification because they do not have natural markings that reliably distinguish between individuals. Waistbands allowed identification without recapturing the animal, but because these bands were subject to loss, we also toe-clipped males following guidelines jointly published by the three North American herpetological societies (*Guidelines for Use of Live Amphibians and Reptiles in Field Research: Applied Ecology Research Group 1987*). We never observed any adverse effects of toe clipping on behaviour, locomotion or mortality, which has been the experience of the majority of studies that have addressed this issue (data and reviews in: Lüddecke & Amézquita 1999; Davis & Ovaska 2001).

### Analysis of Vocalizations

Advertisement call notes have a quasiperiodic fine temporal waveform with a periodicity that varies inversely with body size and corresponds to the fundamental frequency of the call (90–130 Hz), which contains relatively little acoustic energy (Fig. 2). The frequency spectrum of the call consists of a series of harmonics with a bimodal distribution of sound energy. The lower frequency peak is centred between 200 and 400 Hz; a broader, higher-frequency band containing less sound energy occurs between 1000 and 2000 Hz (Fig. 2a, b). We used a Kay DSP Sona-Graph Model 5500 to analyse our recordings. We measured three spectral properties of advertisement calls, including dominant frequency, secondary frequency and the amplitude of the secondary frequency relative to the dominant frequency, from power spectra generated by fast Fourier transformation (FFT) over a 100-ms interval from the middle of a call note, beginning at the point of peak signal amplitude (transform size=1024 points, filter bandwidth=14.5 Hz, 0–4 kHz setting) (Fig. 2a). Similar values were obtained when we averaged the spectrum over the entire duration of a call note. Dominant frequency is the harmonic of single greatest amplitude and corresponds to the second harmonic ( $f_2$ ). We define the secondary frequency as the harmonic of greatest relative amplitude in the higher-frequency band of the bimodal spectrum (i.e. >800 Hz). We determined the amplitude of the secondary frequency relative to that of the dominant frequency in decibels (dB). We converted these logarithmic values to a linear scale ( $\mu\text{bar}$ ) and expressed the amplitude of the secondary frequency as the percentage of relative amplitude of the dominant frequency (0 dB=100%, –6 dB=50%, –12 dB=25%, etc.). We also determined the ratio of secondary frequency to dominant frequency (secondary frequency  $\div$  dominant frequency, hereafter ‘frequency ratio’) as a configurational measure of the distribution of sound energy in the bimodal frequency spectrum. Such a measure is relevant because anurans have two separate hearing organs primarily sensitive to airborne sound, the amphibian and basilar papillae, that are tuned to different frequency ranges (Feng et al. 1975; Lewis et al. 1982).

**Table 1.** Partial correlations from multiple regression of mean values of call properties on snout–vent length (SVL) and water temperature before and after correcting for between-male differences in water temperature for the first recording session†

	SVL		Water temperature	
	Before	After	Before	After
Fundamental frequency	−0.91*	−0.90*	0.33	0.05
Dominant frequency	−0.90*	−0.89*	0.33	0.05
Secondary frequency	−0.16	−0.16	0.03	0.07
Frequency ratio	0.45*	−0.44*	−0.07	0.06
Relative amplitude (%)	−0.33	−0.33	0.00	−0.07
Relative amplitude (dB)	−0.35	−0.35	0.03	−0.05
Note duration	−0.15	−0.18	−0.54*	−0.11
Rise time	0.16	0.15	−0.53*	−0.09
Fall time	−0.44*	−0.43*	−0.27	−0.11
Note period	0.35	0.33	−0.62*	−0.13
Duty cycle	−0.51*	−0.50*	0.24	0.03

†Similar results were obtained for the second recording session (see text).

\* $P < 0.05$ .

The fundamental frequency, which is often absent from the frequency spectrum, is equal to the reciprocal of the waveform periodicity and is related to the perceived ‘periodicity pitch’ of the signal (Simmons & Ferragamo 1993). Using an oscillogram with an expanded time base ( $\pm 0.1$  ms), we measured fundamental frequency ( $f_1$ ; to the nearest 1 Hz) as the reciprocal of the average period of five repetitions of the fine temporal waveform from the middle of a call note (Fig. 2d). We also measured (to the nearest 1 ms) five gross temporal properties from oscillograms ( $\pm 0.8$  ms), including note duration, rise time and fall time (Fig. 2c), as well as note period and duty cycle (note duration ÷ note period) for the first note of all multiple-note calls. Note duration was the time between signal onset and offset. Rise time was defined as the time from signal onset to the time of maximum amplitude; fall time was the time from maximum amplitude to signal offset. Note period was the time between the beginning of the first and second notes in a call. Signal onset and offset were identified as the beginning and ending, respectively, of the repeating fine temporal waveform from an oscillogram with an expanded time base ( $\pm 0.1$  ms). Our recordings were of sufficiently high quality to readily distinguish signal onset and offset from background noise.

Spectral properties of bullfrog advertisement calls vary depending on male body size (Fig. 3a, see below). Hence, size-related differences in spectral properties represent a source of between-male differences that could be important for neighbour–stranger discrimination. Because anurans are ectotherms, temperature can also influence properties of vocalizations (Duellman & Trueb 1986). Temperature coupling in the auditory system can compensate for the effects of variation in temperature on signal variation in some species (e.g. Gerhardt 1978), but the effects of temperature on properties of bullfrog calls and the potential influence of temperature coupling in the bullfrog auditory system have not been determined. Therefore, we analysed both raw and temperature-

adjusted values of call properties. Unfortunately, water temperature and body size (SVL) were correlated in our samples of recordings (first recordings:  $r = -0.15$ ; second recordings:  $r = -0.44$ ). To remove the covariation of call properties with temperature, we combined the data from the two recording sessions and regressed values of each call property on SVL. We next regressed the residuals from this analysis on water temperature. The residuals from this second regression were then added to the predicted values generated by the first regression model. The partial correlations between each call property from the first call note and SVL and water temperature (Table 1) confirm that our correction procedure was generally effective at removing variation due to water temperature while retaining variation due to body size. We used water temperature instead of air temperature because males called exclusively while floating in the water. Fortunately, the correlations between call properties and water temperature were small (Table 1), and analyses based on raw and temperature-adjusted call properties yielded similar results. We used the temperature-adjusted values of call properties in the analyses described below. We obtained similar results in the analyses described below when we analysed the first and second samples of recordings of each male separately and when both recording samples were pooled.

### Patterns of Call Variability

To describe the magnitude of variability in signal properties, we calculated coefficients of variation ( $CV = 100\% \times SD/\text{mean}$ ). We calculated within-male coefficients of variation ( $CV_w$ ) from the variability observed within males during a recording session, and calculated the between-male coefficients of variation ( $CV_b$ ) from the average values of individual males (see Gerhardt 1991). We calculated the ratio of between-male to within-male variability ( $CV_b/CV_w$ ), which serves as a direct measure of the relative magnitudes of variability observed between

and within individuals (Robisson et al. 1993). We also report the average range of variation within individuals as a percentage of individual means, as well as the maximum differences between males as a percentage of the grand mean of all individuals.

We used model II analysis of variance (ANOVA) to examine statistically the variability between males (Sokal & Rohlf 1981; Beecher 1989). Because male bullfrogs commonly produce calls with multiple notes or 'croaks' (1005 of 1078 calls in our samples consisted of multiple notes), there is potential for between-note variability within calls to diminish the magnitude of between-male variability, particularly for gross temporal properties of the amplitude envelope (see Fig. 1). To examine this possibility, we analysed the first, middle and last notes of five multiple-note calls from the first sample of recordings for a subset of 25 individuals. In calls with an even number of notes, we randomly chose one of the two middle notes by flipping a coin. We performed model II ANOVAs in which Male ( $N=25$ ), Call ( $N=5$ ) and Note ( $N=3$ ) were tested as random main effects. For this analysis, we corrected call properties for between-male differences in water temperature separately for first, middle and last notes using the procedures described above. We also performed model II ANOVAs on only the first notes of the same subset of calls. We did not analyse note period and duty cycle because these measures were not available for the last note and contributed little toward statistically discriminating between males (see below). To compare the analyses of first notes and multiple notes, we performed a variance ratio  $F$  test on the between-male variance components based on the mean-square values from the effect of 'Male' for analyses of first notes and multiple notes (Sokal & Rohlf 1981).

We also used ANOVA to compare the within-male and between-male variability of mean values of call properties measured for first notes from the two recordings of individuals made on different dates. We used the results from these ANOVAs to calculate repeatability as the intraclass correlation coefficient (after Sokal & Rohlf 1981; Lessells & Boag 1987).

Unless indicated otherwise, the analyses described below were based on acoustic analyses of single-note calls and the first notes of multiple-note calls. In small choruses or during periods of inactive chorusing, male bullfrogs commonly produce single-note calls. Hence, discrimination between individuals should be possible based on the patterns of variability displayed in a single note.

### Individual Distinctiveness

We used discriminant function analysis (DFA) to examine statistically the individual distinctiveness of advertisement calls (Manly 1994). Discriminant function analysis generates canonical discriminant functions that represent the linear combinations of the original variables that maximally separate individuals in multidimensional signal space (Nelson & Marler 1990). Inspection of the canonical coefficients, which represent the correlations between the discriminant functions and the original variables, determines how variables contribute to

discrimination between individuals. The discriminant functions generated in a DFA can be used to classify each signal as belonging to a particular individual. The percentage of signals assigned to the correct individual serves as a measure of how well the linear combination of variables in the discriminant functions distinguishes between individuals.

We determined classification success for the entire sample of 27 males by calculating the percentage of calls assigned to the correct individual in two ways. First, for each recording session, we used a 'split-data' technique to assign every other advertisement call from an individual to a 'training' data set or a 'test' data set. We analysed each recording sample separately, using the training data set to generate discriminant functions, which we then used to classify calls in the test data set. Second, we used the entire sample of first recordings as a training data set to generate discriminant functions, which we used to classify calls from the second recording session as the test data set. Our classification procedures used prior probabilities that were proportional to the number of calls per male.

Below we report results from discriminant function analyses that included as variables the call properties of fundamental frequency, secondary frequency, frequency ratio, percentage of relative amplitude, note duration, rise time and fall time for the first note of advertisement calls. We excluded dominant frequency and the relative amplitude (measured in dB) from these analyses because they were nearly perfectly correlated with fundamental frequency and the percentage of relative amplitude, respectively (see Table 3). Separate analyses substituting dominant frequency and relative amplitude for fundamental frequency and the percentage of relative amplitude, respectively, yielded similar results, as did analyses that did not exclude these variables. We also excluded note period and duty cycle from our analyses because they were not available for single-note calls. Our results did not differ appreciably in separate analyses that included note period and duty cycle and excluded single-note calls, and these variables were never highly correlated with canonical roots that contributed heavily towards discriminating between males.

Previous studies of territoriality in bullfrogs suggest that no male is likely to have as many as 27 adjacent neighbours at any one time. For example, in the studies by Emlen (1976) and Howard (1978), males commonly had between two and six adjacent neighbours. In our study populations, males most commonly had just two adjacent neighbours and they rarely had more than three (M. A. Bee, unpublished data). Thus, by using a sample size of 27 males, we are potentially overestimating the magnitude of the discrimination problem facing a territorial male. In an effort to tailor our discriminant function analyses to better reflect the task of a bullfrog, we re-examined classification success by considering smaller groups of individuals. We used a resampling procedure to determine the mean percentage of calls assigned to the correct individual for 1000 groups of five males selected randomly with replacement from the larger sample of 27 males.

## RESULTS

Mean values of call properties in our study population (Table 2) were similar to values reported in earlier studies of bullfrog vocalizations (Capranica 1965). Several call properties were correlated with body size (Table 1). Among these, fundamental frequency and dominant frequency were both highly correlated with body size. In the first sample of recordings, for example, SVL accounted for 81% of the variation in fundamental frequency between males (Fig. 3a). There were also several strong correlations between the call properties we analysed (Table 3). Most notably, fundamental frequency and dominant frequency were very highly correlated ( $r_s \geq 0.98$ ), as were our two measures of relative amplitude ( $r_s \geq 0.98$ ).

### Patterns of Call Variability

In Table 2, we report coefficients of variation based on the first sample of recordings for each male. There were no significant correlations between within-male coefficients of variation ( $CV_w$ ) and either SVL, mass, or water temperature ( $|r_s| < 0.37$ ,  $N=27$ , NS). There was, however, a direct relationship between the average  $CV_w$  and between-male variability ( $CV_b$ ;  $r=0.97$ ,  $P < 0.0001$ ; Table 2). For example, for the call property of fundamental frequency, which had the lowest within-male coefficient of variation (mean=1.5%), the majority of between-male differences were small (Fig. 3b; Table 2). Call properties that were more variable within males also tended to vary more between males (e.g. relative amplitude, rise time and fall time; Table 2).

Each of the call properties varied significantly between males, regardless of whether we analysed three notes or only the first note of multiple-note calls (Table 4). There was also significant variation between notes in all call properties except note duration. Between-male variability was generally much greater than between-note variability, with the exceptions of rise and fall times, for which between-note variability was higher. In a multiple-note call, rise times decrease in duration, and fall times increase, proceeding from the first to the last note (Fig. 1). Both analyses yielded similar estimates of between-male variability for all call properties (Table 4), suggesting that analyses of first notes accurately estimate the between-male variability in advertisement calls. An ANOVA based on the first notes of advertisement calls from the first recording sample (539 calls from 27 males) confirmed that all 11 call properties varied significantly more between individuals than within individuals (Table 2).

Between-male variability was significantly greater than the between-recording variability within males for all 11 call properties (Table 2). Mean changes in call properties between the two recording sessions were lowest for properties that showed low within-male variability within a recording session, and highest for call properties that showed high within-male variability. Most call properties had repeatability values similar to those reported in other studies of anuran vocalizations (Table 2; cf. Table 4 in Howard & Young 1998). In general, spectral properties had higher repeatabilities than temporal properties (Table

2). Notice, in particular, the high repeatabilities for fundamental frequency and dominant frequency. These repeatability values exceed previously published values for equivalent properties in the calls of other anurans (see Howard & Young 1998). Our estimates of the repeatability were unaffected by differences in water temperature between the times each male was recorded.

### Individual Distinctiveness

Each of the discriminant function analyses that we performed on the entire sample of 27 males produced either six or seven significant canonical roots. In all cases, four roots had eigenvalues greater than 1.0 and together accounted for 96–98% of the between-male variation. The pattern of differences among the canonical coefficients was highly consistent between analyses, especially for the first three or four canonical roots, which accounted for most of the variation. In Table 5, we summarize the canonical coefficients for the analysis of all 539 calls from the first sample of recordings; results from other analyses were qualitatively similar. Fundamental frequency was the only call property correlated with the first discriminant function, which accounted for 78.3% of the between-male variation. No other call property was correlated with this discriminant function. The correlations between fundamental frequency and the first discriminant function in the other analyses ranged from  $r=0.79$  to 0.86. In separate analyses that substituted dominant frequency for fundamental frequency, the correlations between dominant frequency and the first discriminant function (which accounted for 70.7–76.0% of the between-male variation) ranged from  $r=0.89$  to 0.92. All other correlations between call properties and the first discriminant function were less than  $|r|=0.14$ , and most were below  $|r|=0.10$ . The second discriminant function, which accounted for about 10% of the between-male variation, was correlated with secondary frequency and frequency ratio. The remaining discriminant functions contributed little towards discriminating between males (Table 5).

The mean percentage of calls correctly assigned to individuals using the split-data method ranged between 72.1 and 75.1%. In each analysis, significantly more calls were assigned to the correct individual than expected by chance alone (1 in 27 or 3.7%) (paired  $t$  test:  $t_{26} > 18.4$ ,  $P_s < 0.0001$ ). When we classified the second set of recordings based on discriminant functions generated for the first sample, the percentage of calls classified correctly decreased to 32.8%, but remained significantly higher than expected by chance alone (3.7%) (paired  $t$  test:  $t_{26} = 4.3$ ,  $P = 0.0002$ ).

The mean classification success was higher for smaller groups of five males and ranged from 91.7 to 94.4% when we analysed a single recording sample. The 95% confidence intervals for the smallest and largest mean, respectively, were 91.4–92.1% and 94.1–94.8%. When we used the first set of recordings as the training data set, and the second sample as the test data set, the mean percentage of calls classified correctly was 67.4% (95% CI=66.5–68.4%). We obtained a mean value of 70.3% when we performed

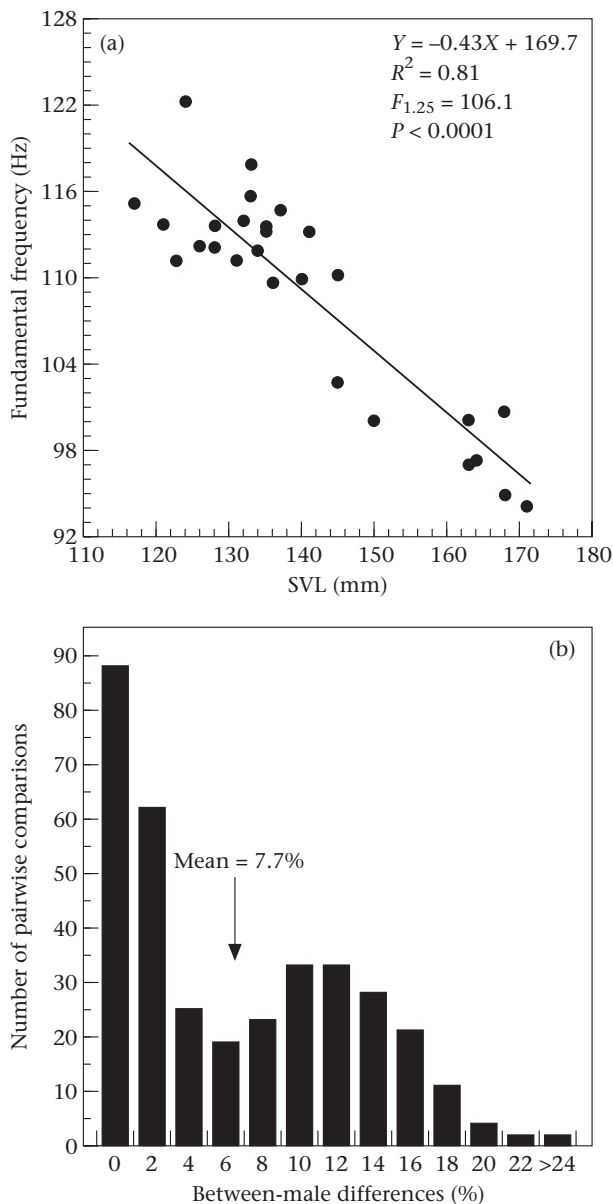
**Table 2.** Means and SDs of call properties and within-male and between-male variation based on the first note of advertisement calls (539 calls from 27 males; 20 calls per male for 26 males, 19 calls from one male)

Call property	Mean	SD	Within-male variability			Between-male variability			Between-recording variability		
			Mean $CV_w$ (range)	Average range of variation within males (% of means)	$CV_b/CV_w$ ratio	Maximum between-male difference (% of grand mean)	$F_{26,492}^\dagger$	Mean difference between recordings (%) <sup>‡</sup>	Mean difference between recordings (%) <sup>‡</sup>	$F_{26,27}^\S$	Intraclass $r$
Fundamental frequency (Hz)	109	8	1.5 (0.6–3.9)	5.8	7.0	4.7	30.5	402.3*	2.2 (0.1–4.9)	29.5	0.93
Dominant frequency (Hz)	219	15	1.5 (0.0–3.0)	4.9	7.0	4.7	30.2	366.0*	2.2 (0.1–4.9)	26.5	0.93
Secondary frequency (Hz)	1301	137	5.6 (0.5–18.1)	18.5	10.5	1.9	63.6	38.9*	8.9 (0.1–28.7)	3.1	0.51
Frequency ratio	6.0	0.7	5.8 (0.4–17.7)	19.7	11.1	1.9	77.2	39.3*	9.7 (0.3–31.1)	2.5	0.44
Relative amplitude (%)	32.2	10.0	21.4 (12.4–41.9)	83.0	30.9	1.4	255.5	33.5*	16.3 (0.5–61.0)	7.1	0.75
Relative amplitude (dB)	-10	+2.3, -3.2	19.1 (5.8–43.3)	73.4	27.0	1.4	211.5	43.3*	14.2 (0.1–46.3)	5.9	0.71
Note duration (ms)	679	60.7	6.6 (3.0–12.9)	24.8	8.9	1.4	65.5	36.1*	6.9 (0.5–22.7)	3.5	0.56
Rise time (ms)	270	48.6	12.6 (6.9–19.2)	49.3	18.0	1.4	119.8	37.6*	12.2 (1.0–36.5)	4.5	0.63
Fall time (ms)	381	52.6	12.4 (5.4–22.9)	46.2	13.8	1.1	98.0	26.6*	10.1 (0.7–36.1)	4.2	0.61
Note period (ms)	1160	105.6	5.6 (2.3–20.9)	22.0	9.1	1.6	107.0	33.9*	5.8 (0.2–28.1)	2.5	0.43
Duty cycle	0.59	0.05	6.2 (1.9–14.3)	24.7	8.0	1.3	78.4	26.5*	5.2 (0.4–17.5)	4.1	0.61

<sup>†</sup>Results of model II ANOVA comparing within-male and between-male variation in the first sample of recordings for 27 males. \* $P < 0.01$ .

<sup>‡</sup>Differences were calculated as a percentage of the smaller of the two mean values for each male from the two recording sessions.

<sup>§</sup>Results of model II ANOVA comparing within-male and between-male variation across the two recording sessions. \* $P < 0.01$ .



**Figure 3.** (a) Regression of the mean fundamental frequency of advertisement calls on snout-vent length (SVL) for the first sample of recordings. (b) Histogram showing the distribution of all pairwise between-male differences in mean fundamental frequency for the first recording session. Pairwise comparisons were calculated as the difference between each male's mean fundamental frequency and the mean fundamental frequencies of all other males' calls based on the first recording sample. Differences in means are expressed as a percentage of the lower value of each pair of means. Numbers below the histogram bars indicate inclusive lower boundaries.

this analysis on calls that were not corrected for differences in temperature. In all cases, the 95% confidence intervals indicated that significantly more calls were classified correctly than expected by chance alone (20%).

The improvements in classification success in the smaller groups of males resulted from a decrease in the overlap between males in multidimensional signal space. In Fig. 4, we plot the means and 95% confidence intervals

for individual male bullfrogs in two-dimensional signal space defined by the first two discriminant functions. There was considerable overlap among males when all 27 males were analysed together. However, the overall degree of overlap was generally reduced when smaller groups of five random males were analysed.

## DISCUSSION

The aims of the acoustical and statistical analyses described here were to investigate the acoustic basis of vocally mediated neighbour-stranger discrimination in bullfrogs. The discriminant function analyses assigned bullfrog advertisement calls to the correct individual significantly more often than expected by chance alone, suggesting that these acoustic signals are individually distinct. All of the acoustic properties examined in our study showed significant between-male variability. Thus all of the call properties that we examined represent potential cues available to territorial bullfrogs for discriminating between neighbours and strangers.

For several important reasons, however, we might expect discrimination to be based on a subset of the signal properties that vary significantly between individuals. First, the auditory system may lack sufficient resolution to perceive the relatively small between-male differences in some call properties. Second, even if auditory resolution is not a limitation, variation in some properties may not exceed the 'just-meaningful difference' (JMD) required to elicit a discriminative behavioural response (Nelson & Marler 1990). Third, because the relative amounts of between-male and within-male variability differed among call properties, we might expect that in discrimination tasks male bullfrogs might weigh most heavily those call properties associated with higher  $CV_b/CV_w$  ratios and  $F$  ratios. Fourth, because not all signal properties propagate equally well through the environment (Wiley & Richards 1978; Richards & Wiley 1980), males should discriminate among individuals using signal properties that propagate with the least attenuation and degradation. Finally, given the high correlations between some call properties, we emphasize that each call property does not uniquely contribute to the total amount of information about individual identity.

Our data overwhelmingly suggest that fundamental frequency and dominant frequency impart an individually distinct voice quality to bullfrog advertisement calls. These two correlated call properties showed the lowest variation within males during single recording sessions, the highest  $CV_b/CV_w$  ratios and highly significant between-male variability, the highest estimates of repeatability between recording sessions, and they were consistently and highly correlated with the first discriminant function, which explained most of the variation among males in the DFAs. Among anuran amphibians (e.g. Gerhardt 1991; Howard & Young 1998), spectral properties generally show low within-male variability, probably due to the morphological constraints imposed by the size and shape of sound-producing structures (Martin 1972). As a result, spectral properties are often highly correlated with male body size (Fig. 3). Hence, the

**Table 3.** Pearson product-moment correlations for call properties that were significantly correlated ( $r_{25} > 0.38$ ) in both the first and second recording sessions

Correlated call properties	First session	Second session
Fundamental frequency and dominant frequency	1.00	0.99
Secondary frequency and frequency ratio	0.77	0.81
Relative amplitude (dB) and relative amplitude (%)	0.98	0.99
Note duration and secondary frequency	-0.45	-0.73
Note duration and frequency ratio	-0.50	-0.78
Note duration and rise time	0.55	0.61
Note duration and fall time	0.60	0.52
Note duration and note period	0.53	0.79
Note duration and duty cycle	0.48	0.70
Fall time and duty cycle	0.40	0.56

**Table 4.** Results of model II ANOVAs examining between-male variability based on multiple notes and the first note and results of  $F$  tests of the ratio of the variance attributable to the effect of Male

	Source	Multiple notes		First note only		Variance ratio	
		$F^*$	$P$	$F_{24,200}$	$P$	$F_{24,24}$	$P$
Fundamental frequency	Male	244.4	<0.01	139.2	<0.01	1.00	0.50
	Call	0.9	0.49				
	Note	114.0	<0.01				
Dominant frequency	Male	242.5	<0.01	139.2	<0.01	0.98	0.52
	Call	1.0	0.43				
	Note	109.9	<0.01				
Secondary frequency	Male	28.8	<0.01	29.9	<0.01	1.34	0.24
	Call	0.5	0.74				
	Note	18.0	<0.01				
Frequency ratio	Male	37.9	<0.01	35.1	<0.01	1.28	0.28
	Call	0.6	0.65				
	Note	5.0	0.01				
Relative amplitude (%)	Male	27.9	<0.01	12.7	<0.01	1.07	0.43
	Call	0.7	0.61				
	Note	17.4	<0.01				
Relative amplitude (dB)	Male	27.9	<0.01	11.7	<0.01	1.05	0.45
	Call	0.7	0.61				
	Note	17.4	<0.01				
Note duration	Male	28.1	<0.01	29.2	<0.01	1.05	0.45
	Call	0.4	0.77				
	Note	0.1	0.89				
Rise time	Male	20.7	<0.01	19.4	<0.01	0.91	0.59
	Call	0.2	0.96				
	Note	79.3	<0.01				
Fall time	Male	8.9	<0.01	6.1	<0.01	1.47	0.18
	Call	0.7	0.62				
	Note	76.3	<0.01				

\*Degrees of freedom: Male (24, 244), Call (4, 344), Note (2, 344).

size-related differences present in fundamental frequency and dominant frequency of bullfrog advertisement calls provides a straightforward mechanism for encoding information about individual identity in acoustic signals.

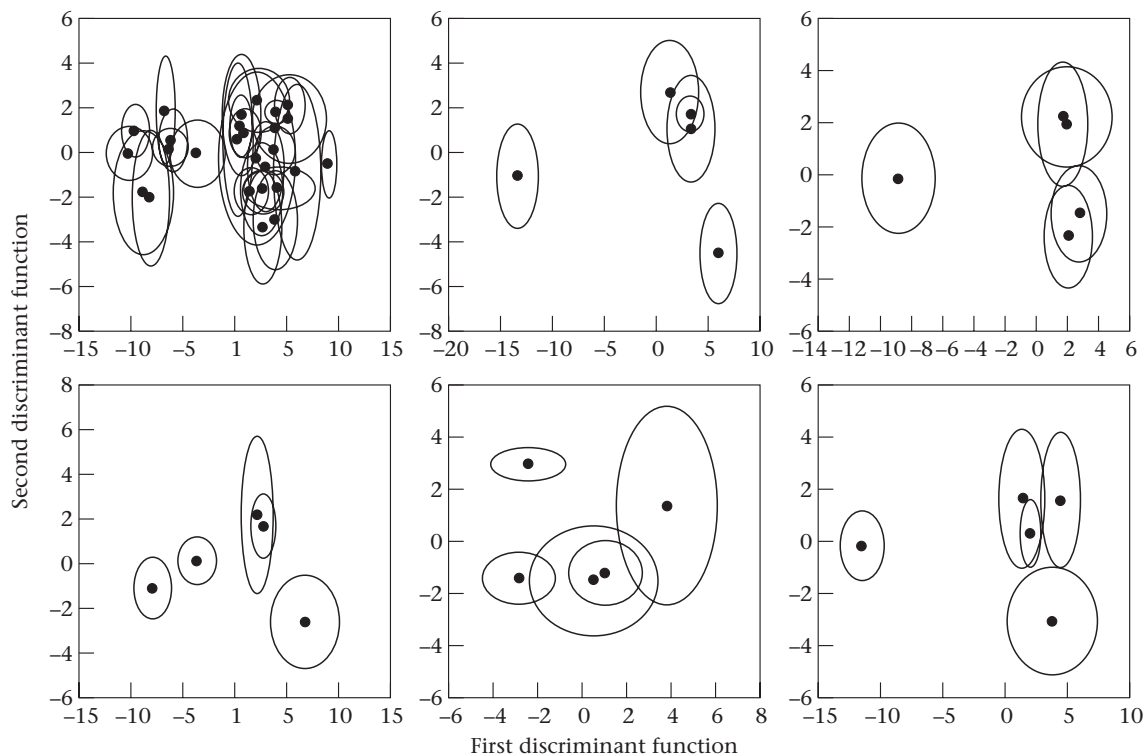
We suggest the hypothesis that territorial male bullfrogs could use the size-related differences in the periodicity of the repeating waveform (i.e. fundamental frequency), low-frequency spectral properties (e.g. dominant frequency), or both to discriminate between some familiar neighbours and unfamiliar males. On the one hand, waveform periodicity and dominant frequency

would be useful recognition cues because both properties propagate well in natural breeding habitat across distances that typically separate territorial neighbours (Boatright-Horowitz et al. 1999). In addition, the bullfrog peripheral auditory system encodes the waveform periodicity or low-frequency spectral components of harmonically complex signals under a wide range of signal-to-noise conditions (Schwartz & Simmons 1990; Simmons et al. 1992, 1993). Hence, the call properties that encode information about individual identity would propagate effectively through the environment and be encoded

**Table 5.** Pooled within-group canonical structure for seven variables used in the discriminant function analysis for the first sample of recordings

	Discriminant function*						
	1	2	3	4	5	6	7
Fundamental frequency	<b>0.79</b>	-0.08	0.16	0.20	-0.07	0.53	0.12
Secondary frequency	0.06	<b>0.74</b>	0.02	0.25	0.60	0.15	0.04
Frequency ratio	-0.14	<b>0.71</b>	-0.01	0.17	0.62	0.23	0.08
Relative amplitude (%)	0.06	-0.12	-0.62	<b>0.74</b>	0.04	0.22	-0.02
Note duration	0.03	-0.52	0.12	0.05	<b>0.78</b>	-0.31	-0.01
Rise time	-0.02	-0.35	0.68	0.59	0.15	-0.12	0.17
Fall time	0.05	-0.22	-0.40	-0.37	0.54	-0.36	0.48
Eigenvalue	31.1	3.7	2.0	1.4	0.9	0.4	0.2
Proportion of variance explained	78.3	9.4	5.0	3.6	2.3	1.0	0.4
Cumulative proportion of variance explained	78.3	87.7	92.7	96.3	98.6	99.6	100

\*The canonical coefficients represented the correlations between the call properties in the model and the discriminant functions. **Bold face** indicates canonical coefficients greater than 0.70.



**Figure 4.** Plots of the advertisement calls of individual bullfrogs in a two-dimensional signal space defined by the first two discriminant functions. Points represent means and ellipses represent 95% confidence intervals for all 27 males (upper left,  $N=539$  calls from the first recording session) and five additional groups of five males selected randomly from the larger sample ( $N=100$  calls from the first recording session).

reliably by the auditory periphery. On the other hand, however, discrimination based only on these properties would probably be far from perfect because of the large number of small between-male differences in fundamental frequency (Fig. 3b). Furthermore, we note that other properties of advertisement calls also have some potential to allow discrimination among male bullfrogs and that discrimination is likely to be based on multiple dimensions of signal variability. Moreover, individual differences in other call properties that were not exam-

ined here, and any reliable individual differences between notes in multiple-note calls, which we also have not examined, could potentially function as recognition cues. Future studies should examine the importance of these other acoustic features as potential recognition cues.

Descriptive analyses of signals are useful for generating testable hypotheses about the properties of communication signals that animals might use to discriminate between conspecifics. This study represents a first step toward understanding how male bullfrogs discriminate

between familiar and unfamiliar individuals based on individual differences in vocalizations. In our companion paper (Bee & Gerhardt 2001a), we report results from a field playback experiment that used the habituation/discrimination paradigm to test the ability of male bullfrogs to discriminate between two signals that differed in fundamental frequency and dominant frequency by a magnitude typical of the between-male differences in the population.

### Acknowledgments

We thank Chris Bowling for assistance in making recordings, Don Martin, Jeff Koppelman and the Missouri Department of Conservation for access to the Little Dixie Conservation Area, Ray Bacon for help in writing the computer code for the resampling procedure and Rick Howard, Vince Marshall, Haven Wiley and an anonymous referee for helpful comments on the manuscript. This research was approved by the University of Missouri IACUC (No. 2944). M.A.B. was supported by an NSF Graduate Research Fellowship, an NSF Doctoral Dissertation Improvement Grant, and an Sigma-Xi Grant-in-Aid of Research. H.C.G. was supported by an NSF grant IBN 9507394 and an NIMH Research Scientist Award. The research presented here was further evaluated and approved by the Animal Behavior Society's Animal Care Committee on 8 August 2001.

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